## 2. INTRODUCTION

### 2.1 Disease Background/Current Treatment

Brain tumors are a major cause of morbidity and mortality in the population. They comprise the third leading cause of death from cancer in persons 15 to 34 years of age (1). Recent evidence indicates that the prevalence of primary brain tumors is increasing, especially in the elderly (2). The astroglial brain tumors, including the highly malignant glioblastoma multiforme (GBM), are the most common primary brain tumors. Despite aggressive therapy, which includes surgical removal of the tumor and postoperative high-dose radiation, the prognosis of patients with GBM is very grim, with a median survival of approximately 10 months (3). When GBM recurs, there is virtually 100% mortality within weeks to a few months. In one study, a mean survival of nine months was found in patients with recurrent GBM who underwent a second operation, but a reasonable quality of life in those patients was limited to 10 weeks following recurrent GBM (5).

### 2.2 Strategy of Gene Therapy

The gene therapy treatment under investigation consists of murine cells producing replication-incompetent retroviral vectors containing the *Herpes simplex* virus thymidine kinase (HSV-Tk1) gene. Integration of the HSV-Tk1 gene into the cellular genome followed by administration of the anti-herpes drug ganciclovir (GCV) leads to the killing of the transduced cells. The mechanism of action is that the thymidine kinase (Tk) produced by the *Herpes simplex* virus can phosphorylate nucleoside analogs, such as GCV, to form nucleotide-like precursors that will block replication of DNA, thereby killing the cell.

A paper published in 1992 suggested the feasibility of *in vivo* HSV-Tk gene transfer for the treatment of malignant brain tumors (6). The central nervous system has several advantages of safety and efficacy for retroviral-mediated *in vivo* gene transfer. First, retroviral vectors integrate and therefore express vector genes only in proliferating cells. In the brain, the tumor is the most mitotically active cell, with only macrophage-derived cells, blood cells, and endothelial cells at minimal risk. Therefore, the possibility of specific transduction of the tumor is enhanced. Second, the brain is a partially immunologically privileged site, which should allow a longer survival of the xenogeneic murine cells and a greater transduction frequency of the growing tumor cells. This feature is further increased since human gliomas are known to further depress local immunity. This is thought to be secondary to a down regulation of IL-2 secretion and diminished expression of high-affinity IL-2 receptors on T-lymphocytes (7). The murine cells should survive sufficiently long in the brain to allow for the transduction of greater numbers of tumor cells. However, this period of survival will be limited since all cells that integrate and express HSV-Tk1 will eventually be destroyed by the ganciclovir treatment or naturally by the immune system. Other advantages of using retroviral vectors include their ability to provide for stable insertion of a non rearranged copy of the gene into the host genome.

A particularly attractive feature of using HSV-Tk1 to sensitize cells to GCV is that in addition to killing the HSV-Tk+ cells when they duplicate, proliferating cells not expressing the HSV-Tk gene but in close proximity to the HSV-Tk transduced cells, are also rendered sensitive to GCV and killed by exposure to the drug. A possible mechanism of action is the transfer of the cytotoxic metabolite, phosphorylated GCV, through cell communication networks such as gap junctions. This phenomenon has been designated the "bystander effect" and is significant because, in principle, it obviates the necessity for transducing every cell in order to eradicate or reduce the tumor. Other mechanisms possibly involved in tumor regression include immunological responses to the murine vector producer cells (VPC) or to tumor antigens. Gene transfer to vascular endothelial cells of vessels surrounding the tumor may also be involved. Destruction of these cells may result in disruption of the blood supply to the tumor leading to necrosis. These potential mechanisms have not been fully characterized and it appears that HSV-Tk/GCV anti-tumor activity is a complex interaction of many systems.

### 2.3 Retroviral-Mediated Gene Transfer

G1Tk1SvNa.7 is a safety modified retroviral vector. The G1 backbone is derived from the Moloney murine leukemia virus (MoMLV). This vector contains a *Herpes simplex* Type I thymidine kinase (HSV-Tk1) gene cDNA that is transcribed from the viral LTR and a bacterial Neomycin resistance ($Neo^R$) gene transcribed from an internal SV40 (simian virus 40) early promoter (LTR—HSV-Tk1—SV—NeoR—LTR). This vector has been modified for increased safety by alteration of the gag start codon to a stop codon and by elimination of viral sequences needed *in trans* for the formation of the virus particle. This has been shown to minimize the potential for the development of replication-competent virus production from producer cells (8,9).

The vectors are packaged by the amphotropic retroviral-vector producer cell line PA317, which is derived from NIH3T3 cells. These vector producer cells are the final product for administration in clinical trials.